COMPOSITION FOR ACCELERATING SECRETION OF ESTROGEN AND REGENERATING TISSUE CELLS OF FEMALE SEXUAL ORGANS

FIELD OF THE INVENTION

The present invention relates to a composition for accelerating secretion of estrogen and regenerating tissue cells of female sexual organs, more particularly, to a composition for accelerating secretion of estrogen and regenerating tissue cells of female sexual organs in postmenopausal women comprising one or more selected from the group consisting of *Platycodi Radix* extract and *Cynanchum wilfordii* extract and/or *Phlomis umbrosa* extract.

BACKGROUD OF THE INVENTION

A hormone secreted from follicles in ovaries, estrogen, develops sexual organs and makes them functional to exhibit the secondary sexual character and accelerate the development of uterus, the proliferation of endometrium, the development of mammary gland and regular menstruation. In addition to ovaries, estrogen is secreted from placenta, adrenal cortex and testis in a small amount. Three types of steroids, estrone, estradiol and estriol found in body are known.

Estrogen is produced from androgenic precursors through an enzymatic process, aromatization. 17 beta-estradiol (E2), a strong estrogen, which exists predominantly in premenopausal

women is synthesized during the formation of follicle, is secreted to blood stream and bound to sex hormone binding globulin in a portion, and then circulates to cells in body. The main metabolic passway of estradiol is to be oxidized reversibly to estrone, a weak estrogen and finally converted to estriol (E3). Estrone is produced by aromatization of androstenedione, precursor of androgen, in the peripheral tissue. The above compounds are metabolized to form sulfate and glucuronide and excreted (Lievertz RW. Pharmacology and pharmacokinetics of estrogen. *Am J Obstet Gynecol* 156:1289-1293(1987)). As aromatization occurs in adipose tissue, those who have many adipose tissues have more estrogen. Estradiol and estrone may be metabolized in liver to estriol, very weak estrogen (Anderson F. Kinetics and pharmacology of estrogens in pre- and postmenopausal women. Int J Fertil 38(suppl 1):53-64(1993)). Other estrogen metabolites as well as estradiol and estrone could function like estrogen. Therefore, it could be understood that the systematic estrogen effects in women depends on both estrogen and its metabolites.

The average concentrations of estradiol and estrone are 520 pg/㎖ and 3,070 pg/㎖ respectively. The peak concentrations of estradiol and estrone are 200-400 pg/㎖ and 170-200 pg/㎖ respectively in ovulation phase and decrease to the minimum concentrations of 40-60 pg/㎖ and 40-60 pg/㎖ respectively in the early stage of menstruation.

The ratio of estradiol to estrone in premenopause is generally lager than 1 (Odonnell MB. Pharmacokinetic and pharmacologic variation between different estrogen products. *J Clin Pharmacol* 35(suppl):18S-24S(1995)). In postmenopause, estrone produced by conversion of adrenal androstenedione becomes a major estrogen.

The metabolic pathway including 2-hydroxyation is more complicated and results in the formation of catecholesterogen. This pathway is more important in the central nervous system such as brain than in the peripheral tissue. Estrogen exhibits its effects by modification of catecholamine metabolite (Lievertz RW. Pharmacology and pharmacokinetics of estrogen. Am J Obstet Gynecol 156:1289-1293(1987)). Since catecholamine interacts with dophamin (a precursor of adrenalin) receptor, alpha 1-adrenalin receptor and serotonin receptor, it is considered to be important. Furthermore, the hydroxy derivatives of estrogen play other roles. For example, 4-hydroxy estrogen functions like estrogen; while 2-hydoxy estrogen does not. However, 2-hydroxy derivatives of estradiol functions not only like estrogen, but also catecholamine (Lievertz RW. Pharmacology and pharmacokinetics of estrogen. *Am J Obstet Gynecol* 156:1289-1293(1987)). This accounts partially for the mechanism how estrogen has an effect on the central nervous system.

The main physiological functions of estrogn is to

regulate the development, differentiation and action of sexual tissues including mammary gland, uterus and ovaries (Kuiper GGJM, Carlsson B, Grandien K, et al. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology* 138:863-870(1997)). Estrogne stimulates the growth of endometrium, myometrium, vagina and urethral epithelium. Estrogen also functions in the smooth flow of blood in sexual tissues, the increase of secretion in uterine gland and induces the expression of progesterone and luteinzing hormone receptor. Estrogen has an effect on the growth and development of backbone, fat distribution in women and lipid metabolite as well as female sexual organs and the secondary sexual character. Estrogen plays roles in skin, collagen tissue, neuron and cardiovascular system.

Estrogen deficiency brings up the symptom such as hat flashes caused from vasomotor instability and in the long term, urogenital degeneration, osteoporosis, tooth loss, arteriosclerosis and coronary heart disease etc, occasionally resulting in dementia (Maddox RW, Carson DS, Barnes CL. Estrogens and postmenopausal women. *U S Pharmacist* 23:141-150(1998); and Guyton AC, Hall JE. Textbook of Medical Physiology. 9th ed. *Philadelphia*: W.B. Saunders, 1996).

In postmenopause, women experience various menopausal symptoms such as blushing and depression caused from the

decreased estrogen secretion in function-deteriorated ovarian. For a hormone replacement therapy, the postmenopausal women lack of estrogen is administered with extrinsic estrogen to reduce the risk of disease occurrence.

Administering estrogen into postmenopausal women improves vasomotion and urogenital diseases, protects and controls osteoporosis and reduces the risk of coronary heart disease (Maddox RW, Carson DS, Barnes CL. Estrogens and postmenopausal women. *US Pharmacist* 23:141-150(1998)).

However, the problem is that such a hormone replacement therapy may increase the activity of cancer-inducing gene, leading to increased incidence rate of breast cancer and metrocarcinoma, etc.

The known estrogen-replacing agents are artificially synthesized or available from natural source. For example, premarin made from horse urine increases the concentration of estradiol and estrone up to that in menstrual phase (Stumpf PG. Pharmacokinetics of estrogen. *Obstet Gynecol* 75(suppl):9S-14S(1990)). However, as the source of the product is horse urine, the patient compliance is far poor.

As the example of plant products, mexico yam contains steroid precursor used for synthesizing esterified-estrogen (estrogen sodium salt mixture comprised of estrone 75%-85% and equiline 6%-15% as main components) (e.g., estratab, Menest). Such plants as soy bean, date palm, pomegranate contain

nonsteroid plant compound, phytoestrogen. Natural estroen originated from these plant functions as agonists and/or antagonist (Barrett J. Phytoestrogens: friends or foes? *Environ Health Perspect* 104:478-482(1996)).

17 beta-estradiol, estrone, estrogen sulfate are the exmples of chemically modified estrogen. Such synthesized estrogen is used generally as an oral contraceptive, but hardly in hormone replacement therapy.

In short, the amount of estrogen secreted decreases with aging and the pattern of estrogen secretion is directly related to the advancement of aging. It has been recognized in the art that the administration of hormone-replacing agents allows to improve the usual symptom of menopause, while the conventional hormone-replacing agents give rise to severe adverse-effects.

Throughout this application, various patents and publications are referenced and citations are provided in parentheses. The disclosure of these patents and publications in their entities are hereby incorporated by references into this application in order to more fully describe this invention and the state of the art to which this invention pertains.

DETAILED DESCRIPTION OF THIS INVENTION

The present inventors have made intensive study to overcome the defects of estrogen-replacing agents used in conventional hormone replacement therapy. As a result, the inventors have found that a composition comprising one or more

selected from the group consisting of *Platycodi Radix* estract and *Cynanchum wilfordii* extract and/or *Phlomis umbrosa* extract gives rise to regeneration and proliferation of tissue cells of female sexual organ by the increase of estrogen concentration in serum resulting from the induction of estrogen secretion.

Accordingly, it is an object of this invention to provide a composition for accelerating estrogen secretion.

Other objects and advantages of the present invention will become apparent from examples to follow, appended claims and drawings.

In one aspect of this invention, there is provided a composition for accelerating estrogen secretion, which comprises one or more selected from the group consisting of *Platycodi Radix* extract and *Cynanchum wilfordii* extract as active ingredient.

According to the present invention, a natural source, *Platycodi Radix* is meant to a root of *Platycodi Radix* (Jacq.)A. DC. belonging to perennial plant, *Campanulaceae*, and contains not only tens types of saponin glycosides such as platicodion-A, C, D1 and D2, platicodigenin, but also polygalacine-D1 and D2 in addition to betulin, inutin and phytosterol. It has been known that *Platycodi Radix* exhibits expectoral, antitussive, anti-inflammatory and antipyretic efficacy.

Additionally, a raw material, *Cynanchum wilfordii* is a perennial plant belonging to *polygonaceae* with height of 1-3 m. Its root is tuberous and thick. It contains cinancotoxin and phytotocotoxin and used as tonics

The present inventors have screened various natural sources to search material capable of increasing estrogen concentration in blood by accelerating estrogen secretion and found that the extracts obtained from *Platycodi Radix* or *Cynanchum wilfordii* have such activity.

According to a preferred embodiment of this invention, the composition of the present invention comprises further the extract obtained from *Phlomis umbrosa*.

Phlomis umbrosa used in the present invention is a perennial plant belonging to *Labiatae* with height of 70-150 cm. It has 5 tuberous roots. Its leave is edible and its root and rhizome is used for medicines. *Phlomis umbrosa* extract of the present invention protects ovaries and accelerates estrogen secretion (see the Korean Unexamined Pat. Publication No. 1998-61480).

Furthermore, the present inventors found that the composition comprising *Platycodi Radix, Cynanchum wilfordii* and *Phlomis umbrosa* extracts exhibits synergic effect on /accelerating estrogen secretion.

The extracts of *Platycodi Radix, Cynanchum wilfordii* and *Phlomis umbrosa* used as active ingredients in the present

invention exhibit excellent efficacy in accelerating estrogen secretion without adverse effects such as cancer occurrence, compared with conventional estrogen-replacing agents.

The extracts of *Cynanchum wilfordii* and *Phlomis umbrosa* used in the present invention may be provided from various organs and tissues (e.g., stem, leave, root, fruit and seed, etc) of *Cynanchum wilfordii* and *Phlomis umbrosa*, and the most preferable extract is that obtained from root thereof.

The extracts from *Platycodi Radix*, *Cynanchum wilfordii* or *Phlomis umbrosa* are obtained using various extraction solvents: (a) water, (b) absolute or water-bearing lower alcohol containing 1-4 carbon atoms (methanol, ethanol, propanol, butanol, etc.), (c) mixture of lower alcohol and water, (d) acetone, (e) ethyl acetate, (f) chloroform, (g) 1,3-butylene glycol and (h) butyl acetate. Most preferably, the extracts of this invention is obtained using water as extraction solvent. Furthermore, it is apparent to one skilled in the art that other conventional solvents may be employed for substantially identical extraction efficiency.

The extracts of this invention include those subject to additional purification by the well-known methods in the art as well as those obtained by extraction with above solvents. For instance, it could be appreciated that active fractions obtained using a variety of additional purification methods such as an ultrafiltration with defined molecular weight

cut-off value and various chromatography (designed for purification dependent upon size, charge, hydrophobicity and affinity) are included in the present extracts.

Preferably, the extracts of this invention are active fractions obtained by ultrafiltration membrane with low molecular weight cut off range, more preferably by extracting *Platycodi Radix* and *Cynanchum wilfordii* with hot water, whereby a crude extract is obtained; and filtering the crude extract by means of ultrafiltration membrane with molecular weight cut off of 50,000-100,000 and most preferably by means of ultrafiltration membrane with molecular weight cut off of 100,000. The extracts of this invention can be obtained in the form of powder by use of vacuum distillation, lyophilization or spray drying.

The composition of the present invention for accelerating estrogen secretion prevents the degeneration of female sexual organ by the regeneration or proliferation of various tissue cells of female sexual organs such as ovary, uterus, vagina and oviduct.

According to a preferred embodiment of the persent invention, the amount of *Platycodi Radix, Cynanchum wilfordii* or *Phlomis umbrosa* extracts in the composition of this invention as active ingredients is 20-80% by weight based on the total amount of composition, more preferably 25-30 wt%, and most preferably 27 wt%. According to the composition

comprising more than two of the extracts, the amount of the extracts is 20-80% by weight based on the total amount of composition.

According to a preferred embodiment of this invention, the composition of the present invention may further comprise one or more of calcium, arginin and lysine. Calcium plays the secondary role in preventing osteoporosis by strengthening the bones of women in menopause, being necessary for muscle motion and involved in neural transmission, blood coagulation, activation of hormones and enzymes, anti-inflammation and alleviation of insomnia. Arginin, an essential amino acid needed for the synthesis and degradation of growth hormone, is involved in muscle development, removal of lipids, regeneration of cell and immune-boosting. Lysine is helpful in body growth, calcium-absorption and biosynthesis of collagen, antibodies and enzymes. Such additional ingredients could elevate the efficacy of the present plant extracts.

Meanwhile, the composition of the present invention comprising one or more selected from the group consisting of *Platycodi Radix* extract and *Cynanchum wilfordii* extract and/or *Phlomis umbrosa* extract shows the efficacy *in vivo* such as induction of estrogen secretion and regeneration or proliferation of tissue cells of female sexual organ.

In another aspect of this invention, there is provided

a pharmaceutical composition for treating or preventing a disorder associated with menopause, which comprises a pharmaceutically effective amount of one or more of *Platycodi Radix* and *Cynanchum wilfordii* extracts; and a pharmaceutical acceptable carrier.

In addition, a pharmaceutical composition of the present invention may further comprise *Phlomis umbrosa* extract as active ingredient.

A pharmaceutical composition of the present invention comprising a pharmaceutically effective amount of one or more selected from the group consisting of *Platycodi Radix* extract and *Cynanchum wilfordii* extract and/or *Phlomis umbrosa* extract is useful for treating or preventing a disorder associated with menopause developed after menopause. Such disorders include fever due to poor vasomotion, blushing, night sweating, climactic diseases, osteoporosis, diseases in genitourinary system, cardiovascular diseases, dementia, tooth loss and collagen loss.

A pharmaceutical composition of the present invention comprising a pharmaceutically effective amount of one or more selected from the group consisting of *Platycodi Radix* extract and *Cynanchum wilfordii* extract and/or *Phlomis umbrosa* extract is advantageous to efficient and radical treatment of disorder associated with menopause, since it can accelerate estrogen secretion *in vivo*.

A pharmaceutical composition of the present invention has much less adverse effects, particularly the incidence of cancers such as metrocarcinoma and breast cancer than conventional hormone replacement therapy.

In the pharmaceutical compositions of this invention, the pharmaceutically acceptable carrier may be conventional one for formulation, including carbohydrates (e.g., lactose, amylose, dextrose, sucrose, sorbitol, mannitol, starch, cellulose), gum acacia, calcium phosphate, alginate, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, water, salt solutions, alcohols, gum arabic, syrup, vegetable oils (e.g., corn oil, cotton-seed oil, peanut oil, olive oil, coconut oil), polyethylene glycols, methyl cellulose, methylhydroxy benzoate, propylhydroxy benzoate, talc, magnesium stearate and mineral oil, but not limited to. The pharmaceutical compositions of this invention, further may contain wetting agent, sweetening agent, emulsifier, buffer, suspending agent, preservatives, flavors, perfumes, lubricant, stabilizer, or mixtures of these substances.

A pharmaceutical composition of this invention may be administered orally or parenterally. For parenteral administration, intravenous injection, subcutaneous injection, intramuscular injection, nasal spray, sublingual

spray may be employed. Preferred method is oral administration or sublingual spray and the most preferred is oral administration.

The correct dosage of the pharmaceutical compositions of this invention will be varied according to the particular formulation, the mode of application, age, body weight and sex of the patient, diet, time of administration, condition of the patient, drug combinations, reaction sensitivities and severity of the disease. It is understood that the ordinary skilled physician will readily be able to determine and prescribe a correct dosage of this pharmaceutical compositions. According to a preferred embodiment of this invention, where the pharmaceutical compositions is orally administered, suitable dosage unit for human host is to administer once a day with the composition containing 0.1-5 g of *Platycodi Radix*, *Cynanchum wilfordii*, or *Phlomis umbrosa* extract, the most preferred with the composition containing 2 g of the extracts.

According to the conventional techniques known to those skilled in the art, the pharmaceutical compositions of this invention can be formulated with pharmaceutical acceptable carrier and/or vehicle as described above, finally providing several forms including a unit dosage form. Non-limiting examples of the formulations include, but not limited to, a solution, a suspension or an emulsion, an extract, an elixir, a powder, a granule, a tablet, a capsule, emplastra, a liniment,

a lotion and an ointment.

In further aspect of this invention, there is provided a food composition for accelerating estrogen secretion, which comprises one or more selected from the group consisting of *Platycodi Radix* extract and *Cynanchum wilfordii* extract as active ingredient.

In addition, a food composition of the present invention may further comprise *Phlomis umbrosa* extract as active ingredient.

In the food composition of this invention, it can comprise typical ingredients incorporated into food products known to one skilled in the art. For example, for preparation of dirnks, citric acid, liquid fructose, sucrose, glucose, acetic acid, malic acid, fruit juice, *Eucommiae Cortex* extract, *Zizyphus jujuba* extract and *Glycyrrhiza, Liquorice* extract may be further included in addition to *Platycodi Radix, Cynanchum wilfordii* or *Phlomis umbrosa* extract. A food of the present invention is very effective in treating or preventing a disorder associated with meonpasue caused by reduced estrogen secretion level.

A composition of the present invention not only accelerates estrogen secretion and regenerates or

proliferates tissue cells of sexual organs, but also has much less adverse effects than those chemically synthesized since it comprises *Platycodi Radix* and *Cynanchum wilfordii* extracts and/or *Phlomis umbrosa* extract used traditionally as oriental pharmaceuticals.

BRIF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph representing the average estrogen concentration in serum of rats administered with the composition of this invention for each group;

Fig. 2a is a graph representing the average weight of ovaries and uterus of rats administered with the composition of this invention for each group; and

Fig 2b is a graph representing the average relative weight of ovaries and uterus of rats administered with the composition of this invention for each group.

The following specific examples are intended to be illustrative of the invention and should not be construed as limiting the scope of the invention as defined by appended claims.

EXAMPLES

MATERIALS

Oriental pharmaceuticals of *Platycodi Radix*, *Cynanchum*

wilfordii and *Phlomis umbrosa* were purchased in Gyeongdong Market (Seoul, Korea) and used for preparing extracts thereof

EXAMPLE 1: PREPARATION OF COMPOSITION CONTAINING *Platycodi Radix* extract

EXAMPLE 1-1: Preparation of Low Molecular Weight-Extract of *Platycodi Radix* Using Ultrafiltration Membrane

To 50 g of dried *Platycodi Radix*, 1.5 liters of distilled water were added, followed by heating for 2 hr over room temperature for extraction, thereby obtaining aqueous extract. The extract was concentrated to final volume of 500 ㎖.

The extract was centrifuged at 3000 rmp for 10 min, after which for filtration the supernatant obtained was passed through ultrafiltration membrane with molecular weight cut off (MWCO) of 100,000 by means of stirred cell apparatus (purchased from Amicon, USA). The nitrogen gas pressure used was fixed at 3 atm. The extract was dried to give powder of which the amount is shown in Table 1 below.

Table 1

Step	Weight (g)	Extraction Efficiency (%)
Raw materials	50	100
Extract	14	28
Filtration with MWCO of 100,000	11.5	23

EXAMPLE 1-2: Preparation of Composition for Oral

Administration

The composition for oral administration containing *Platycodi Radix* extract was prepared with ingredients as in Table 2.

Table 2

Ingredients	Amount (Weight%)
Platycodi Radix extracts	50
Calcium from marine algae	24
Arginine	16
Lysine	10

EXAMPLE 2: PREPARATION OF COMPOSITION FOR ORAL ADMINISTRATION CONTAINING *Cynanchum wilfordii*

The extract from root of *Cynanchum wilfordii oral* and the composition for oral administration were prepared in the same manner as in Example I-1 and I-2. The amount of the extract is shown in Table 3. The ingredients of oral composition of this invention are shown in Table 4 below.

Table 3

Step	Weight (g)	Extraction Efficiency (%)
Raw materials	50	100
Extract	15	30
Filtration with MWCO of 100,000	12.1	24.2

Table 4

Ingredients	Amount (Weight%)
Cynanchum wilfordii extract	50
Calcium from marine algae	24
Arginine	16
Lysine	10

EXAMPLE 3: PREPARATION OF COMPOSITION FOR ORAL ADMINISTRATION CONTAINING *Platycodi Radix*, *Cynanchum wilfordii* and *Phlomis umbrosa* extracts

The extract from 50 g of *Platycodi Radix*, 25 g of root of *Cynanchum wilfordii* and 25 g of root of *Phlomis umbrosa* and the oral composition were prepared in the same manner as in Example I-1 and I-2. The amount of the extract is shown in Table 5. The ingredients of oral composition of this invention are shown in Table 6 below.

Table 5

Step	Weight (g)	
	Platycodi Radix	*Cynanchum wilfordii* and *Phlomis umbrosa*
Raw materials	50	50
Extract	14	36
Filtration with MWCO of 100,000	11.5	30

Table 6

Ingredients	Amount (Weight%)
Platycodi Radix, *Cynanchum wilfordii* and *Phlomis umbrosa* extracts	50
Calcium from marine algae	24
Arginine	16
Lysine	10

EXAMPLE 4: EFFICACY TEST OF COMPOSITION OF THIS INVENTION USING RAT

To examine the effect of composition of this invention, the compositions prepared as in Example 1, Example 2 and Example 3 were administered into rats. The concentration of estrogen in blood and the weight of sexual organ were measured in time course and anatomical and pathohistological test were performed.

EXAMPLE 4-1: Oral Administration of Composition

A portion of left ovaries of 70 female rats weighted out 300 g with age of 51 weeks was removed to induce decrease in secreting amount of estrogen. Rats were sorted on their weight to distribute equally in weight into groups comprising 10 rats in a group. They were randomly distributed to 7 groups (G1, G2, G3, G4, G5, G6 and G7). 100 mg/kg of composition of Example 1-2 for G2, 1000 mg/kg of composition of Example 1-2 for G3, 100 mg/kg of composition of Example 2 for G4, 1000 mg/kg of

composition of Example 2 for G5, 100 mg/kg of composition of Example 3 for G6, and 1000 mg/kg of composition of Example 3 for G7 were dissolved and suspended in distilled water acceptable to injection and administered orally in 10 mg/kg. Distilled water for injection as a control was administered to G1. For administration, rats were fixed and the composition was directly injected into their stomachs using metal sonde for oral administration. 10 days after the removal of ovaries, injection was done and continued for 5 weeks in once a day and 7 days per week.

EXAMPLE 4-2: Measuring Estrogen Concentration in Serum

Rats fasted overnight prior to dissection were dissected 8 weeks after administration. Blood was collected from postcaval vein and stood at room temperature for 15 min for coagulation. Then, serum was separated by centrifuging at 3,000 rpm for 10 min. The separated serum was kept in -70℃ and analyzed with 1470 wizard γ counter (Perkin Elmer Life Science). Quantification of estrogen concentration in rat serum was made with EIA (enzyme immunoassay) Kit (DSL, US). The results are shown in Table 7.

Table 7

Group	Estrogen Concentration in Serum (ng/mℓ)	
	Average	Standard Deviation.
G1	135.6	36.74
G2	156.9	45.22
G3	158.6	46.35
G4	154.2	50.09
G5	157.1	48.46
G6	158.7	51.17
G7	160.9	44.37

As shown in Table 7, the estrogen concentration in serum increased depending on injected amount in the groups administered with the composition of Example 1 containing *Platycodi Radix* extract and in the groups administered with the composition of Example 1 containing *Cynanchum wilfordii* extract. Groups administered with the composition of Example 3 containing *Platycodi Radix*, *Cynanchum wilfordii* and *Phlomis umbrosa* extracts showed higher estrogen concentration than groups administered with the composition of other Examples.

Accordingly, the composition containing *Platycodi Radix* extract and composition containing *Cynanchum wilfordii* extract increased estrogen concentration in serum by accelerating the estrogen secretion in female rats. In addition, the composition containing *Platycodi Radix*, *Cynanchum wilfordii* and *Phlomis umbrosa* extracts showed synergy effect on accelerating estrogen secretion.

Example 4-3: Weighing Ovaries and Uterus

Rats in each group were dissected 8 weeks after administration and left ovaries and uteri were weighed with electronic balance (BP310S, Sartorius). The results are shown in Table 8.

Table 8

Absolute Weight of Organs (Average, g)							
Group	G1	G2	G3	G4	G5	G6	G7
Ovary	0.0659	0.0635	0.0695	0.0633	0.0687	0.0639	0.0699
Uterus	0.6901	0.6920	0.7989	0.6911	0.7902	0.6663	0.8181
Relative Weight of Organs (Average, % body weight)							
Group	G1	G2	G3	G4	G5	G6	G7
Ovary	0.0228	0.0229	0.0235	0.0223	0.0230	0.0227	0.0244
Uterus	0.2425	0.2445	0.2757	0.2344	0.2703	0.2370	0.2857

As shown in Table 8, absolute weight of ovaries and uteri and their relative weight to body weight increased depending on the injected amount in the groups administered with the composition of Example 1 containing *Platycodi Radix* extract of this invention and in the groups administered with the composition of Example 2 containing *Cynanchum wilfordii* extract of this invention.

Groups administered with the composition of Example 3 containing *Platycodi Radix*, *Cynanchum wilfordii* and *Phlomis umbrosa* extracts showed higher increase in absolute weight of

ovaries and uteri and their relative weight to body weight.

Accordingly, it could be understood that the composition comprising *Platycodi Radix* extract and composition containing *Cynanchum wilfordii* extract proliferate and regenerate tissue cells of sexual organ by accelerating estrogen secretion in female rat. In addition, the composition containing *Platycodi Radix*, *Cynanchum wilfordii* and *Phlomis umbrosa* extracts showed synergy effect on growth of cells.

Example 4-4: Anatomical, Pathohistological analysis

Groups administered with the composition of Example 3 and control group were dissected to analyze anatomical characteristic with naked eye. The results showed that uterus was expanded correlating to the injected amount since the expansion of uterus was observed in 2 rats of control group, G1, 3 rats of G6 administered with 100 mg/kg and 5 rats of G7 administered with 1000 mg/kg. Moreover, black ovarian cyst was not observed in control group, but in 2 rats of G6 and 1 rat of G7.

The rats of above groups were fixed with 10% neutral formalin solution and trimmed. Their paraffin embedding blocks were prepared, microtomed and H&E-stained. And then, pathohistological analysis was done with optical microscope. The results are shown in Table 9.

Table 9

Organ	Symptom	No. of rat individual		
		G1	G6	G7
Uterus	Proliferation of epithelium cell in endometrium	3	7	4
	Vesiculation of uterine gland in endometrium basal layer	5	8	8
	Expansion	1	2	6
	Proliferation of endometrium basal layer	0	0	1
Ovary	Vesiculation upon cysts expansion	3	5	4
Vagina	Proliferation of mucosal membrane epithelium cell of vagina	5	6	7

As shown in Table 9, the pathohistological analysis demonstrated that the expansion of endometrial cavity and other changes related to proliferation were observed in relatively higher frequency in the groups administered with compositions of this invention, compared with the control group. Therefore, it is recognizable that the composition of this invention can accelerate estrogen secretion, resulting in several histological alterations associated with the growth of tissue cells from sexual organ.

Having described specific examples of the present invention, it is to be understood that such examples are only

preferred embodiments and should not be construed as limiting the scope of the invention. Therefore, the substantive scope of the invention may be determined by appended claims and their equivalents.

As described previously, the present invention provides a composition, a pharmaceutical composition and a food composition capable of replacing estrogen, which comprise one or more selected from the group consisting of *Platycodi Radix* extract and *Cynanchum wilfordii* extract and/or *Phlomis umbrosa* extract. The composition of this invention comprised of the extracts of natural source shows little or no adverse effects such as cancer occurrence compared with estrogen-replacing agent used for conventional hormone replacement therapy; in addition, it has excellent effect on the regeneration and proliferation of tissue cells of female sexual organs, so that it is applicable to treatment of various disorders and diseases developed in postmenopausal women who are deficient in estrogen. Moreover, the composition of this invention has little impurities and high amount of active ingredients.